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A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY
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PLANT PHYSIOLOGY
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LIGHT AND PIGMENT DEVELOPMENT IN THE KIDNEY BEAN[1]

ROBERT A. WHITMORE

(WITH ONE FIGURE)

For more than one hundred years investigators have worked on the effect of light upon growth and development of plants. Much of this early work, reviewed by MACDOUGAL (**6**), was qualitative only, but it was established by this work that variation in quantity and quality of light could bring about pronounced morphological and physiological changes in plants. Work in this field since 1900, reviewed by BURKHOLDER (**2**), has tended in the direction of better measurement and control of both the quality and quantity of light and toward separation of the morphological and physiological effects of light. For such studies light of so low intensity that photosynthesis is negligible is desirable.

This paper is one of a series from this laboratory on the effects of low light intensity on plant growth. The first paper in this series was presented by WITHROW (**9**), who found that 100 ergs per square centimeter per second of yellow-green light induced the greatest morphological response in kidney bean seedlings. He found the least effect to be produced by blue and near infra-red light. WITHROW found that in the absence of radiation the major portion of the reserve material translocated from the cotyledons remained in the vicinity of the cotyledons, in the hypocotyls and in the first internodes, and that radiation in the red and yellow end of the spectrum increased the amount of translocation and a larger proportion of the translocated material was moved to the epicotyls and roots. He concluded that this effect was not directly related to chlorophyll synthesis.

The second paper in this series was presented by BIEBEL (**1**) and was a study of the effect of radiation of wavelength longer than 5850 A upon the development of kidney bean seedlings. He found that the morphological effect of radiation is dependent upon the intensity of radiation, with the effect decreasing at a decreasing rate with increase in intensity of incident radiation, and that morphological response varied according to the manner in which the energy of radiation is increased. BIEBEL also found that the

[1] Third of a series supported by the DR. WALLACE C. and CLARA A. ABBOTT MEMORIAL FUND of the University of Chicago.

Q_{10} of the response is about one, and that albino maize seedlings gave the same response as normal seedlings.

Methods

The present paper presents the first part of an investigation into the physiological response of green plants to low intensity radiation and is an investigation of the effects of different regions of the visible spectrum upon chloroplast pigment development. Low light intensity, of the order of 300 ergs per square centimeter per second, was used so that light would be the limiting factor in growth and development of the plant. Hence it was necessary to use a plant with a large supply of reserve food in the seed, and for convenience in analysis it was desirable that the plant used develop rapidly and that the seedlings be rather large. Further, in order that the results of this investigation be comparable to the results of the two previous papers in this series the same plant should be used. For these reasons the red kidney bean, *Phaseolus vulgaris,* was selected as the test plant.

To determine the spectral regions of greatest interest for this study the light absorption of leaves of plants grown in the greenhouse was determined.

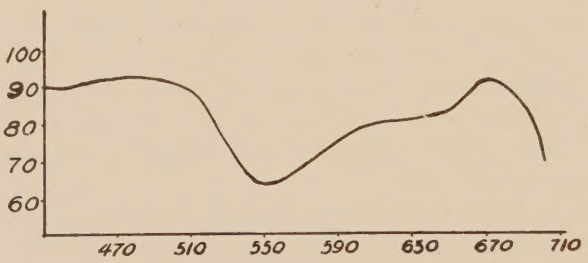

FIG. 1. Light absorption, in percentage, plotted against wavelength in millimicrons, of trifoliate bean leaves.

The first trifoliate leaves, approximately three-quarters full size, were removed from five bean plants grown in soil in the greenhouse during the latter part of September, a period of fair light intensity, 2,000 to 3,000 foot candles at noon. The light absorption of each of the leaves was determined at intervals of 100 A from 4300 to 7000 A, using a Keuffel and Esser spectrophotometer, the results averaged, and plotted (fig. 1). The variation in light absorption of any leaf from the average was small and within the limits of error of the apparatus used. Two regions of maximum absorption were noted, one in the blue end of the spectrum centered at 4900 A, and one in the red end centered at 6700 A. A well marked minimum was noted, centered approximately at 5500 A. Light absorption amounted to approximately 90 per cent. in the regions of maximum absorption, and to approximately 60 per cent. in the region of minimum absorption.

For the purpose of this work it was considered desirable to isolate relatively narrow spectral regions centered, as nearly as possible, in the regions of maximum and of minimum absorption of the leaves. An investigation

was made of light sources and it was decided to use bright line sources having one, or several, bright lines in the desired regions. An inclosed arc mercury arc lamp, manufactured by the General Electric Company, proved suitable for the blue and green regions, with bright lines at 4358 and 5461 A. A neon-gas-filled, low-pressure-discharge lamp, made in the form of a flat spiral and excited by a 7,000-volt transformer was used as a source of red light. This lamp was made to order by a neon sign manufacturing company.

Both the mercury arc lamps and the neon lamp had bright line emission spectra in regions other than that desired and the mercury lamps had in addition a continuous spectrum; it was thus necessary to combine the light source with a light filter to produce approximately monochromatic illumination. Commercial light filters in the required size, 20×20 inches, and with the required light absorption characteristics were found to be not available, or very expensive.

The best solution of the filter problem seemed to be the use of dyed gelatin filters. A paper by HNATEK (**4**) and one by WITHROW (**10**) provided information on the construction and use of such filters. It was necessary, however, to make a spectrophotometric examination of a number of organic dyes to find some with light absorption characteristics suitable for construction of the desired filters. A number of red, orange, yellow and purple dyes were found which could be used to locate the short-wave absorption limits of gelatin filters in nearly any desired region of the visible spectrum. Very few dyes were found which combined high absorption for red and high transmission for blue or green light. Fortunately two dyes, Fast Turquoise Blue 8 GL and Napthol Green, were found to absorb red light and, in combination with other dyes, permitted construction of the desired filters.

Construction of the filters was simple after selection of the proper dyes. A sheet of double strength, first quality window glass twenty inches square was washed with soap and water, rinsed with distilled water, and carefully leveled in a room reasonably free from dust and drafts. Thirty grams of gelatin and ten grams of sorbitol, added as a plasticizer at the suggestion of WITHROW (**10**), were added to 250 ml. of water in a 600-ml. beaker. This solution was melted on a water bath and the required amount of dye dissolved in 250 ml. of water was added with careful stirring, to avoid air bubbles as much as possible. After cooling to slightly below 60° C., the solution of 500-ml. total volume was poured onto the sheet of glass. The gelatin-dye solution was carefully spread with a stirring rod and any air bubbles were broken by touching with the finger, if necessary moistened with alcohol. No difficulty was experienced with the solution running off the glass if it was clean and level and if the solution was below 60° C. The filters were allowed to stand at room temperature until the film was hard and dry, and were then durable and permanent.

To isolate the 4358 A line of the mercury arc 1.0 gm. of Fast Turquoise Blue 8 GL and 0.25 gm. of Methyl Violet 4B were dissolved in 250 ml. of water and added to the gelatin solution as described above. The 5461 A line

was isolated with 1.0 gm. of Light Green SF, 1.25 gm. of Napthol Yellow, and 0.25 gm. of Napthol Green dissolved in 250 ml. water and added to the gelatin solution. Two separate filters were necessary for isolation of the 6700 A region of the neon lamp. One filter contained 0.50 gm. of Methyl Violet, the other contained 0.375 gm. of Basic Fuchsin plus 1.00 gm. of Orange G. Two filters were necessary as precipitation occurred if all three dyes were mixed solution.

A primary filter consisting of ten centimeters of distilled water provided with a cooling coil through which tap water was circulated was used between the light sources and the gelatin filters.

The effectiveness of the filter systems was determined by assembling the light-source-filter sytem in position one meter above the seed bed and operating it continuously for seven days. The radiation was then examined visually with a Bausch and Lomb constant deviation spectrometer for the presence of radiation other than that from the desired bright line, and especially for the presence of other bright lines. For the systems used no evi-

TABLE I

RADIATION CHARACTERISTICS OF TEST PLOTS

PLOT	LIGHT SOURCE	WAVELENGTH	INTENSITY	TIME OF ILLUMINATION
		A	*ergs/sq. cm/sec*	*hr.*
1	Hg arc, 400-watt	4358	360	16.6
2	Hg arc, 250-watt	5461	300	16.0
3	Neon tube	6678, 6717 6598	240	16.3
4	Dark	.....	.....	.....
5	Incandescent lamp	4000 to 7000	300	16.0

dence of radiation other than that desired was found. The absence of ultraviolet radiation was checked by observing a 0.1 per cent. solution of quinine sulphate for fluorescence.

The intensity of the radiation was measured with a thermopile and galvanometer circuit similar to that used by BURNS (**3**). In operation the voltage of the thermopile was balanced to zero by a bucking voltage generated by current flowing through a resistance common to both circuits. The bucking voltage was adjusted by suitable resistances and the current measured on a 0–30 microammeter with multiplying shunts. At balance the voltage from the thermopile was equal to the IR drop in the common resistance. With a common resistance of the order of one-tenth ohm this circuit proved to be well adapted to measuring low intensity radiation. The thermopile-galvanometer circuit was calibrated against a standard lamp from the National Bureau of Standards, in ergs per square centimeter, and the response was linear over the range used.

Radiation incident upon the seed bed of each plot was adjusted to as near equal quanta intensity as possible by varying the density of the filters.

Slight differences from the desired intensity were corrected by varying the time of illumination so that in each 24-hour period each plot received equal quanta of energy.

Five plots were used for growing the plants with the radiation characteristics given in table I.

One hundred seeds were planted in each plot (except number five) in a gravel bed $20 \times 20 \times 4$ inches, saturated with tap water by a subirrigation system once every 48 hours. Plot number five, used for pigment comparison only, was planted with fifty seeds in sand and watered manually.

Results

Three weeks after planting the seedlings were harvested. Eighty plants were collected from each plot, those showing abnormal growth or development were discarded. At this time the cotyledons were shriveled but were still attached to the stem. The seedling lengths averaged 35.7 cm. in plot number one, 36.4 in two, 32.5 in three, and 41.3 cm. in plot number four. The leaves were removed, weighed, an aliquot taken for dry weight determination, and the pigments extracted from the remainder by the method of SCHERTZ (7). The chlorophyll, carotene, and carotenol content of the leaves in terms of milligrams of pigment per gram fresh weight of leaf tissue was determined by the method of LOOMIS and SHULL (5). These data are presented in table II.

TABLE II

MILLIGRAMS PIGMENT PER GRAM FRESH WEIGHT LEAF TISSUE

RADIATION	PLOT	CHLOROPHYLL	CAROTENE	CAROTENOL
		mg.	*mg.*	*mg.*
Blue	1	0.177	0.019	0.098
Green	2	0.723	0.040	0.103
Red	3	0.532	0.016	0.175
Dark	4	0.008	0.021	0.176
Total	5	1.045	0.048	0.320

The total weight of leaves developed by 80 plants varied from 10.0 to 22.7 gm. in different plots. The total pigment produced per plot by eighty plants has been calculated and presented in table III to show the effects of radiation on pigment development per plant.

TABLE III

TOTAL PIGMENT, IN MILLIGRAMS, AND LEAF WEIGHT IN GRAMS

PLOT	LEAVES		CHLOROPHYLL	CAROTENE	CAROTENOL
	WET WT.	DRY WT.			
	gm.	*gm.*	*mg.*	*mg.*	*mg.*
1	16.20	2.62	2.86	0.312	1.58
2	15.82	2.82	11.42	0.638	1.63
3	22.70	3.14	12.05	0.363	3.96
4	10.0	1.34	0.08	0.210	1.76

As the molecular ratios of the pigments have more physiological significance than the weight ratios, the molecular ratios are presented in table IV.

TABLE IV

MOLECULAR RATIOS OF CHLOROPLAST PIGMENTS PER PLOT

PLOT	CHLOROPHYLL/ CAROTENE	CHLOROPHYLL/ CAROTENOL	CAROTENOL/ CAROTENE
1	5.5 (1)	1.15 (1)	4.8 (2)
2	10.7 (2)	4.4 (4)	2.4 (1)
3	19.9 (4)	1.93 (2)	10.3 (4)
4	.....	.....	7.9 (3)

Assuming, from WILLSTÄTTER and STOLL (**8**), that the molecular ratio of chlorophyll *a* to *b* is 3/1, a semi-quantitative examination of the chlorophyll extracts indicated that this ratio is approximately correct for the plants used, the mean molecular weight of chlorophyll is 895.5. The molecular weight of carotene is 536 and that of carotenol is 568. The mol ratio of chlorophyll/carotene is then 536 × weight of chlorophyll divided by 895.5 × weight of carotene; that of chlorophyll/carotenol is 568 × weight of chlorophyll divided by 895.5 × weight of carotenol; and that of carotenol/carotene is 536 × weight of carotenol divided by 568 × weight of carotene.

The initial dry weights of the seeds of the eighty plants harvested; the dry weight minus the seed coat weight; the wet and dry weights of the harvested plants; the ratio, in percentage, of dry to wet weight; the loss in dry weight; and the percentage loss in dry weight are given for each plot in table V.

TABLE V

WET AND DRY WEIGHTS, IN GRAMS, OF SEEDS AND OF HARVESTED PLANTS

PLOT	SEEDS		HARVESTED PLANTS				
	DRY WT.	MINUS SEED COAT	WET WT.	DRY WT.	DRY/WET	LOSS	LOSS
			gm.	*gm.*	%		%
1	37.3	33.7	244.8	12.8	5.24	20.9	62.1
2	37.7	34.3	233.8	12.1	5.19	22.2	64.7
3	37.9	34.5	214.6	11.6	5.42	22.9	66.4
4	37.4	33.7	274.2	12.0	4.38	21.7	64.5

Table VI shows the effect of the radiation used on development of the plant. The dry weight ratios, in percentages, of the plant organs to the whole plant, and the top/root ratios are given in table VII.

Discussion

It is evident, from tables VI and VII, that a maximum movement of reserve materials from the cotyledons and hypocotyls occurred in the plants grown in red light and that relatively more of the translocated material moved to the roots in these plants. Plants grown in blue light had less total

TABLE VI

EFFECT OF RADIATION ON DEVELOPMENT OF PLANTS

PLANT PART	RESULTS	PLOT			
		1	2	3	4
		BLUE	GREEN	RED	DARK
Roots	Wet wt. (gm.)	37.0	30.8	36.4	24.4
	Dry wt. (gm.)	1.9	2.0	2.0	1.1
	Dry/wet (%)	5.1	6.5	5.5	4.5
	Length $\frac{\bar{x}}{s}$	8.6 1.57	6.9 1.53	7.5 1.36	6.2 1.17
Hypocotyls including cotyledons	Wet wt. (gm.)	136.8	128.8	111.8	169.7
	Dry wt. (gm.)	6.7	6.2	5.9	7.4
	Dry/wet (%)	4.9	4.8	5.3	4.4
	Length $\frac{\bar{x}}{s}$	22.2 2.46	20.8 2.89	18.5 2.24	26.3 3.53
Epicotyls	Wet wt. (gm.)	71.0	74.2	66.4	80.2
	Dry wt. (gm.)	4.2	3.9	3.7	3.5
	Dry/wet (%)	5.9	5.3	5.6	4.4
	Length $\frac{\bar{x}}{s}$	13.6 1.46	15.8 1.96	14.0 1.76	15.0 3.07

translocation of material from the hypocotyls and cotyledons, and less material translocated to the roots than the plants grown in the red plot, while plants grown in the green plot were intermediate between the red and blue plots with respect to translocation of reserve material. In the absence of light the greater portion of the reserve material remained in the region of the hypocotyls and cotyledons and the roots received less material than in any of the irradiated plots. These results are in good agreement with those of WITHROW (**9**).

The effect of the radiation used upon respiration is evident from table V. The maximum loss of weight occurred in plants grown in red light and the minimum loss of weight in plants grown in blue light, while the loss of weight by the plants grown in green light was approximately equal to the loss in weight of the plants grown in darkness. The extent to which respiratory stimulation by light was compensated by photosynthesis is unknown, but it may be noted that plot number one, irradiated with blue light, contained approximately one-fourth the amount of chlorophyll as plots two and three (table III) but had the least loss in weight. Light absorption was also at a

TABLE VII

PERCENTAGE DRY WEIGHT AND TOP/ROOT RATIOS

	BLUE	GREEN	RED	DARK
Root (%)	14.8	16.5	17.2	9.16
Hypocotyl* (%)	52.4	51.2	50.8	61.7
Epicotyl (%)	32.8	32.2	31.9	29.2
Top/Root (ratio)	6.44	5.05	4.81	9.93

* Plus cotyledons.

minimum in this plot as the leaves were small and undeveloped; it may be mentioned also that light absorption by the carotenoid pigments is very strong in this region of the spectrum. These data indicate that the efficiency of photosynthesis is much greater at 4358 A than at 5461 or at 6700 A, or that respiratory stimulation is much less at the shorter wavelengths of radiation. Work was in progress to measure the photosynthetic and respiratory effect in these plots, using normal and albino maize plants, but was interrupted early in 1942.

The effect of the radiation used upon pigment development is evident from tables II and III. As might be expected, the entire spectrum (table II, plot 5) proved most effective in development of the greatest amounts of chloroplast pigments, on a fresh weight of leaf tissue basis. Plants grown in green light contained approximately $\frac{3}{4}$ as much chlorophyll as those grown in the total spectrum; those in red light about $\frac{1}{2}$ as much; and those grown in blue light approximately $\frac{1}{6}$ the chlorophyll content of those grown in the complete spectrum. The small amount of chlorophyll found in plants grown in darkness apparently developed during collection, manipulation, and extraction as a second set of plants grown in the dark and collected and extracted with a minimum of light exposure contained no chlorophyll. The maximum carotene content was found in plants grown in the total visible spectrum; about $\frac{4}{5}$ this amount in plants grown in green light; about $\frac{1}{3}$ this amount in plants grown in red light; and nearly an equal amount, about $\frac{1}{2}$ of that developed in the total spectrum, was found in plants grown in blue light and in darkness. Carotenol was found in greatest quantity in the plants grown in the complete spectrum. Plants grown in red light and in darkness contained nearly the same amount of carotenol, about $\frac{1}{2}$ the amount contained in plants grown in the complete spectrum. Plants grown in blue and in green light were approximately equal in carotenol content and contained about $\frac{1}{3}$ the amount of plants grown in the complete spectrum. All of these comparisons are made on the amount of pigment per gram fresh weight of leaf tissue of the respective plants.

As leaf development under the different wavelengths of radiation was quite different another basis for pigment comparison was also used (table III). In this table the total pigment developed per plot of 80 plants is listed. As the plants grown in the total spectrum were grown under conditions differing considerably from those in the first four plots no attempt was made to include these plants in this table. On the basis of total pigment developed per 80 plants those grown in red light contained the maximum amount of chlorophyll with nearly as much in those grown in green light. Plants grown in blue light contained only about 0.23 as much chlorophyll as those grown in red radiation. The carotene content was at a maximum in the green plot with plants in the blue and red containing about $\frac{1}{2}$ the carotene content of those in the green plot. Plants grown in darkness contained the least carotene, about $\frac{1}{3}$ as much as those in the green plot. The carotenol content was greatest in plants grown in red light; about 0.40 this amount in

plants grown in blue and in green light; and about 0.45 this amount in plants grown in darkness.

The molecular ratios of the chloroplast pigments developed in each plot were calculated (table IV). The chlorophyll/carotene ratio, expressed as the nearest integral number, was 1, 2, and 4 for plants grown in blue, green, and red light, respectively; the chlorophyll/carotenol ratio was 1, 4, and 2 for plants grown in blue, green and red light; and the carotenol/carotene ratio was 2, 1, 4, and 3 for plants grown in the blue, green, red, and dark plots.

Summary

Red kidney bean seedlings were grown in gravel in tap water under monochromatic illumination and in darkness. The wavelengths and the intensity of the illumination was 4358, 5461, and 6700 A, and 360, 300, and 240 ergs per square centimeter per second. The seedlings were harvested and the lengths, wet weight, and dry weight of the roots, hypocotyls, and epicotyls were determined. The leaves were removed and analyzed for chlorophyll, carotene, and carotenol.

A maximum loss of weight was observed in plants grown in red light and a minimum loss of weight in plants grown in blue light. The weight loss of plants grown in green light was approximately equal to that of plants grown in darkness. No measurements were made of respiration or photosynthesis. Calculation of dry weight ratios indicated that maximum translocation of reserve material occurred in plants grown in the red light and that the proportion of such material translocated to the roots was larger than in plants grown in green or in blue light. The least translocation of reserve material was found in plants grown in darkness and the proportion of such material remaining in the region of the hypocotyl and cotyledons was a maximum in these plants.

On a fresh-weight basis, of the leaves, the maximum amount of chlorophyll and carotene was developed by plants grown in the green plot; the maximum amount of carotenol was found in the plants grown in darkness and was approximately equal to that developed by the plants grown in red light. The minimum chlorophyll content was developed in the plants grown in blue light, of the three irradiated plots, and the minimum carotene was found in the red, and the minimum carotenol in the blue plots. When the total pigment developed by 80 plants in each plot was calculated the maximum chlorophyll and carotenol was found in plants grown in the red plot, excluding the dark plot from chlorophyll comparisons, and a maximum carotene content in the green plot. The minimum chlorophyll and carotenol content was found in the plants grown in the blue plot, and the minimum carotene was developed in the plants grown in the dark. The molecular ratios of the chloroplast pigments developed per plot of 80 plants were determined, but insufficient data were obtained to evaluate their meaning in terms of the physiology of the plants.

The writer wishes to express his gratitude to Dr. C. A. Shull for his guidance and advice during the course of this project.

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